Reproductive parameters of the commercially exploited deep-water shark, *Deania calcea* (Centrophoridae)

by

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ABSTRACT. - Little information is available on the reproductive parameters of the commercially exploited deep shark *Deania calcea* (Lowe, 1839). Samples were obtained from deep-water longlines operating at depths around 1450 m in the Portuguese continental slope between September 2008 and July 2009. Both the male and female reproductive tracts were morphologically and histologically analysed. Females were more numerous than males, which may be related to possible sex segregation by depth/geographical area and/or fishermen preference for females (larger and heavier). Reproductive indices (gonadosomatic index, oviducal gland width) as well as the fecundity and length at first maturity were estimated. The ovarian and the gestation period showed asynchronous cycles. The analysis of both the gonadosomatic index and the oviducal gland width showed evidence for the existence of two periods of high ovulation (March and September). The ovarian fecundity ranged from 7 to 17 follicles while the uterine fecundity ranged from 3 to 5 embryos. The length at first maturity estimated for females was 96.6 cm. In total 72 embryos were sampled, with total length varying between 96 and 309 mm; embryos considered to be fully developed presented the external yolk sac totally depleted and ranged between 251 and 309 mm.

RÉSUMÉ. - Paramètres de reproduction du requin de profondeur *Deania calcea* soumis à exploitation commerciale.

Très peu d'information est disponible sur les paramètres de reproduction du requin de profondeur *Deania calcea*, qui est commercialement exploité. Les échantillons ont été obtenus à partir des débarquements commerciaux de palangres posées sur le talus continental du Portugal à des profondeurs d'environ 1450 m entre septembre 2008 et juillet 2009. Les paramètres de reproduction des mâles et des femelles ont été analysés morphologiquement et histologiquement. Les femelles sont plus abondantes que les mâles et cette situation pourrait être liée à une ségrégation par la profondeur ou la région géographique et/ou une préférence des pêcheurs pour les femelles (plus grandes et plus lourdes). Les indices de reproduction (index gonadosomatique, épaisseur de la glande de l'oviducte), ainsi que la fécondité et la longueur à la première maturité sexuelle ont été estimés. Le cycle ovarien et la période de gestation ont montré des cycles asynchrones. L'analyse des indices gonadosomatiques et de l'épaisseur de la glande de l'oviducte a montré la possible existence de deux périodes marquées d'ovulation (mars et septembre). La fécondité ovarienne varie de 7 à 17 follicules alors que la fécondité utérine varie de 3 à 5 embryons. Pour les femelles, la longueur à la première maturité est de 96,6 cm. Au total, 72 embryons ont été observés, d'une longueur totale variant entre 96 et 309 mm. Les embryons proches de la fin du développement présentent un sac vitellin externe totalement résorbé et ont une longueur totale comprise entre 251 et 309 mm.

Key words. - Centrophoridae - *Deania calcea* - Atlantic NE - Reproductive cycle - Fecundity - Length at first maturity - Histological analysis.

Sharks are extremely vulnerable to overfishing because of their low reproductive rates, high length at first maturity and direct relationship between stock and recruitment (Carrier *et al*., 2004). Therefore many shark fisheries need the implementation of a management plan that focuses on the sustainable use of the resource (Zhou and Griffiths, 2008). However, development of fishery management plans can be difficult when there is a lack of adequate biological information and appropriate fisheries data (Cortés, 1998; Chang and Liu, 2009).

Life history traits are among the biological parameters important for assessment purposes and among them the study of reproductive parameters is fundamental for understanding/predicting population dynamics, as well as for improving the management of the assessed stock and for stock identification studies (Neves *et al*., 2009).

The study of deep-water sharks and deep-water fisheries in general involves additional difficulties due to the sampling and depth constraints. The fast development of these fisheries has not been followed by monitoring programs to obtain information on the species biology and population dynamics, a significant limitation in deep-water sharks making them particularly vulnerable to fishing pressure (Hamlett, 2005). However, and despite the few information on the biology of these species, there are already some papers published on reproductive parameters namely on the histological characterization of the different organs of the reproductive trait (e.g., Hamlett and Hysell, 1998; Hamlett *et al*., 1998)

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Cybium 2011, 35(2): 131-140.

or the assessment of reproductive indices (e.g., Yano, 1991; Capapé *et al*., 1999; Clarke *et al*., 2002; Capapé *et al*., 2008; Figueiredo *et al*., 2008).

Deania calcea (Lowe, 1839), the birdbeak dogfish (Chondrichthyes: Squalidae) occurs in the Atlantic from Iceland to South Africa, and in the Pacific, off Japan, Taiwan, Australia, New Zealand, Peru and Chile (Compagno *et al*., 2005).

In European waters, the birdbeak dogfish is captured as a by-catch of deep-water fisheries that started to be exploited during the early 1960's (Gordon, 2001), when the traditional fisheries on the continental shelf declined (Koslow *et al*., 2000). In Portugal, birdbeak dogfish is a by-catch of the longline fishery targeting black scabbardfish (*Aphanopus carbo* Lowe, 1839) and there is little information on its biology. The landings of *D. calcea* were around 120 tonnes/year in the last decade (DGPA, 2010). However, this data should have taken into account that since 2005, a total allowable catch (TAC) has been allocated in European waters (Council Regulation (EC) No 2270/2004) to deep-water sharks, a group that includes 11 species. In this context fishermen prefer to land the species with the highest commercial value such as *Centrophorus granulosus* (Bloch & Schneider, 1801), *Centrophorus squamosus* (Bonnaterre, 1788) and *Centroscymnus coelolepis* Barbosa du Bocage & de Brito Capello, 1864. Therefore, and since 2005, birdbeak dogfish is only landed when the above mentioned species are captured in fewer quantities or when the birdbeak dogfish specimens are larger and weigh more. As a result of this TAC, catch data for birdbeak dogfish are underestimated since there is a high percentage of discards that, unfortunately, is unknown but should be quantified.

The main goal of this paper is to provide information on the reproductive strategy of this deep-water species, including the reproductive cycle, fecundity and size at maturation.

MATERIALS AND METHODS

Sampling

Birdbeak dogfish were captured with deep-water longlines at the Portuguese continental slope, between September 2008 and July 2009, at depths around 1450 m, and samples were obtained from landed specimens in Sesimbra fishing harbour. The total length (TL, ± 1 mm), total weight (TW, ± 50 g), sex and maturity stage (according to the criteria described in table I) of each individual were registered. In mature females, egg diameter (Ø, ± 1 mm) and egg weight (EW, ± 0.01 g) were also recorded; in pregnant females, the sex of each embryo was recorded as well as its TL (± 1 mm) and TW (± 0.01 g). The three structures of the female reproductive tract (ovary, oviducal gland and uterus) and the testis were removed, weighted (± 0.01 g) and stored in 10% formaldehyde solution buffered at pH = 7 with carbonates (CH_2O; M = 30.03; D = 1.02 g/cm^3). Tissue samples were dehydrated in a graded ethanol series (70-96%) and embedded in methacrylate resin. Two 3 μm sections were cut from each slice and stained with toluidine blue.

Reproductive parameters

The reproductive parameters analysed included the sex ratio, the gonadosomatic index (GSI), the oviducal gland width index (OGWI), the ovarian (OF) and uterine (UF) fecundities and the length at first maturity (Y).

Sex ratio was tested by the chi-square test for both adults and embryos and the Mann-Whitney test was applied to test the existence of significant differences between sexes and the influence of the total length according to sex. The GSI (percentage of the total weight of gonad/total weight of the individual), was determined for males and females and the OGWI (expressed in percentage of the oviducal gland width/total weight of the individual) was determined for females in the maturity stage 3. To investigate if there was seasonality in the reproductive events, the monthly evolution of the GSI (immature and mature individuals in the ovarian cycle) and OGWI were also analysed.

Fecundities (OF and UF) were estimated based on the mean of the total number of fully developed follicles in maturity stage 3 females and on the total number of embryos in maturity stages 5 and 6 females, respectively. The Y was estimated based on the proportion of mature females and males by length class (class interval = 2 cm, due to the low representativeness of certain length classes) and applying the following logistic model: $Y = 1 / \{1 + e^{[-1 \times (a + b \times TL)]}\}$. The coefficient of correlation (r) was also adjusted to the logistic equation.

For statistical analysis, ANOVA was applied to the data that presented normality and homogeneity of variances (evolution of males GSI by maturity stages). Whenever data did not meet those assumptions the nonparametric Kruskal-Wallis test was applied (evolution of females GSI by maturity stages).

RESULTS

Sex ratio

A total of 615 specimens were sampled (459 females and 156 males) with a sex ratio of 2.94:1, which was significantly different from 1:1 ratio ($\chi^2 = 149.28$; $p < 0.0001$). For most months, females predominated over males. The females were larger than the males, ranging from 67.7 to 121.1 cm and 64.7 to 91.5 cm, respectively. The Mann-Whitney test revealed significant differences between sexes, regarding TL (U = 5459.500; p-level < 0.001).

Characterization of reproductive tract

The female reproductive tract consists of three main organs (ovaries, oviducal gland and uterus), with the maturation cycle divided into two phases: ovarian and uterine, according to whether the main events occur in one or another structure (Tab. I).

Regarding the ovary, the ovarian follicle appears fully formed at 0.40 mm in diameter and all follicular layers are already present: theca externa, theca interna, basement membrane, granulosa and vitelline membrane, from the outer to the inner region (Fig. 1A). From that size and until the ovarian follicles reached the final maturation (with a diameter of 54 mm) the differences among the oocytes are mainly due to an increase in thickness of the granulosa and vitelline layers and basal membrane. Furthermore, in the granulosa layer a change from cuboidal to columnar cells was observed in larger follicles (Fig. 1B).

The oviducal gland did not show the typical four region zonation (club, papillary, baffle and terminal), common in most elasmobranchs, because the papillary zone was absent. The oviducal gland, which was not present in immature females, increased in size until the final development of the ovarian phase was reached and the lamellae and tubules were particularly evident at that stage. The lamellae increased in size as the oviducal gland grew and this increase was most evident in the uterine phase, particularly during pregnancy and postpartum. Tubules also suffered an increase in size while growing in a spiral form. The tubules were completely full of secretion products at the end of ovarian phase (Fig. 1C) but during pregnancy and postpartum, the tubules were almost empty and a decrease in its spiralling form became evident.

The uterus was analysed according to the type of epithelium, the development of the muscle layers and connective tissue, as well as the shape and size of uterine villi. The epithelium showed an evolution through the maturation development: the cuboidal cells became columnar, and there was a differentiation of the *lamina propria* of the epithelium. In

Table I. - Macroscopic maturity scales for males and females *Deania calcea* used in this study and with the new terminology suggested by Brown-Peterson *et al*. (2011).

		Males		
		This paper		Brown-Peterson *et al*. (2011)
	1	Juvenile	Testes thin, with a linear form. Flexible and short claspers.	Immature
	2	Maturing, adolescent	Testes with a linear form, but starting the segmentation. Claspers more robust but flexible, longer than the margin of the pelvic fin.	Developing
	3	Mature (Adult)	Testes segmented. Claspers rigid and long.	Spawning capable
	4	Active	Testes segmented and highly vascularized. Claspers more flexible than in stage 3, and dilated.	Actively spawning capable
		Females		
		This paper		Brown-Peterson *et al*. (2011)
Ovarian stages	**1**	Juvenile	Ovary with small follicles (diameter < 10 mm). Uterus narrow. Oviducal gland undifferentiated.	Immature
	2	Maturing, adolescent	Follicles becoming differentiated to various sizes. Oviducal gland present. Uterus narrow.	Developing
	3	Mature (Adult)	Follicles with large dimensions (> 13 mm).	Spawning capable
Uterine stages	**4**	Developing	Embryos present in the uterus but not distinguished.	Actively spawning capable
	5	Differentiating	Small embryos are distinguished with no pigmentation.	
	6	Expecting	Embryos more or less fully formed, pigmented and can be sexed easily.	
	7	Spent	Ovary present atretic follicles. Uterus is enlarged and extremely vascularized.	Regressing stage
Ovarian stage	**8**	Maturing (not for the first time)	Ovary with follicles in many stages of development and high frequency of atretic follicles. Uterus enlarged and thicker than stage 2.	Regenerating stage

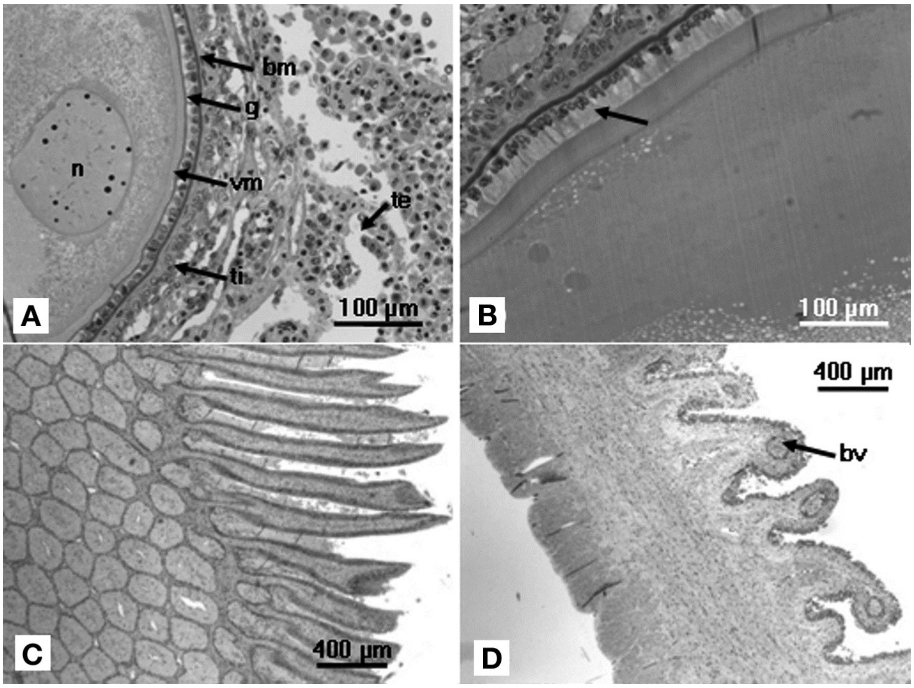

Figure 1. - **A**: Ovarian follicle, 0.787 mm in diameter; **B**: Ovarian follicle, 1.933 mm in diameter with columnar granulosa cells (arrow); **C**: General view of tubules and lamellae at the end of the ovarian phase; **D**: Uterine villi at the end of the ovarian phase. bm: basement membrane; bv: blood vessel; g: granulosa; n: nucleus; te: theca externa; ti: theca interna; vm: vitelline membrane.

post-partum, the basal lamina invaded the *lamina propria* of the epithelium which indicated that the epithelium was in a dissociation phase.

The muscular layer was composed of a typically transverse tissue. An increase in this layer was observed during pregnancy to allow the enlargement of the uterus and, in the postpartum period, the muscle layer reduced its size. The associated connective tissue layer was very flexible, vascularized and incorporated with a large amount of fibroblasts and collagen fibbers. This tissue presented its greatest dimension during the gestation period.

The shape and size of uterine villi reached their maximum development at the gestation stage (Fig. 1D). Uterine villi were much vascularized, showing two central blood vessels of larger caliber and peripheral capillaries; secreting cells were also present in this peripheral region.

The testes of *D. calcea* presented a diametric or linear pattern because the development of spermatocysts (embedded in the testicular matrix or connective tissue) occurred along its cross-section. Spermatocysts included both germ and somatic cells (Sertoli cells) and during spermatogenesis the following different stages of germ cells were identified: spermatogonia (primary and secondary), spermatocytes (first and second order), spermatids (primary and secondary), and spermatozoa (immature and mature). In the first stage of development spermatocysts presented the nuclei of Sertoli cells lining the lumen, while the germ cells (spermatogonia) occurred at a more peripheral position (Fig. 2A). In the second stage of development, a migration of the nuclei of Sertoli cells towards the peripheral region occurred, and the lumen of the spermatocyst was still visible (Fig. 2B). Primary spermatocytes constituted the next step of spermatogenesis and were characterized by their large round nuclei containing elongated chromosomes. The Sertoli cells maintained their peripheral location while the spermatocyst lumen became poorly evident (Fig. 2C). Secondary spermatocytes resulted from the division of primary spermatocytes and were characterized by a reduction of the nuclei diameter which nevertheless maintained their spherical shape; a better individualization of the boundaries of the Sertoli cells could be observed (Fig. 2D). Spermatocysts containing immature spermatids were the next step of development and were characterized by the elliptical nuclei (Fig. 2E). Mature spermatids (Fig. 2F) elongated and formed loose bundles. Immature spermatozoa were characterized by the formation of fewer loose bundles although the heads maintained a peripheral position in the spermatocysts (Fig. 2G). The final step of development corresponded to the mature spermatozoa that presented even tighter bundles. They were located nearer the lumen thus putting in evidence the Sertoli cell cytoplasm with a granular appearance (Fig. 2H). After being released, the empty spermatocysts kept the Sertoli cells in their peripheral position and thus putting in evidence their smooth reticulum (Fig. 2I).

Reproductive cycles

Based on macroscopic and microscopic observation of the reproductive tract of female birdbeak dogfish, eight maturity stages were characterized (Tab. I). The ovarian and the uterine cycles were characterized as asynchronous. Females in stage 1 and 8 were present in all samples. Stage 2 was present almost all-year round (in very low percentage) except in October and March. Stage 3 females were found in a low percentage over the sampling period, except in December when they reached ca 30% of the total. Females in the uterine cycle (stages 4 to 7) were poorly represented throughout the year; the pregnant stage 4 was not sampled during October, November and January, while pregnant stage 6 was not captured in November and May (Tab. II).

Regarding the male reproductive cycle (Tab. II), the information gathered was less than the one obtained for females and no males were sampled in December, April, June and July. A four stage sexual maturation scale was established (Tab. I) based on the different development stages of the sexual cells and/or different percentage of the area occupied by each cell type. Thus, males of stage 1 (immature) had narrow and uniform testes characterized by the presence of spermatogonia (48%) and spermatocytes (52%). Immature males were found in all months, except in September, February and March. Stage 2 (maturing) was characterized by an enlargement, increased segmentation and irrigation of the testes. Internally, they were characterized by the presence of all cell types although spermatocytes (43%) and spermatids (27%) were present in larger percentages. Maturing males were present in all months and corresponded to the total number of males sampled in February and March. In stage 3 (adult) males were characterized by being sexually active and therefore presented a higher percentage of spermatozoa (20%). Stage 4 (active) was characterized by a lower percentage of spermatozoa and a larger amount of empty cysts could also be observed. Males in stage 3 and 4 were underrepresented.

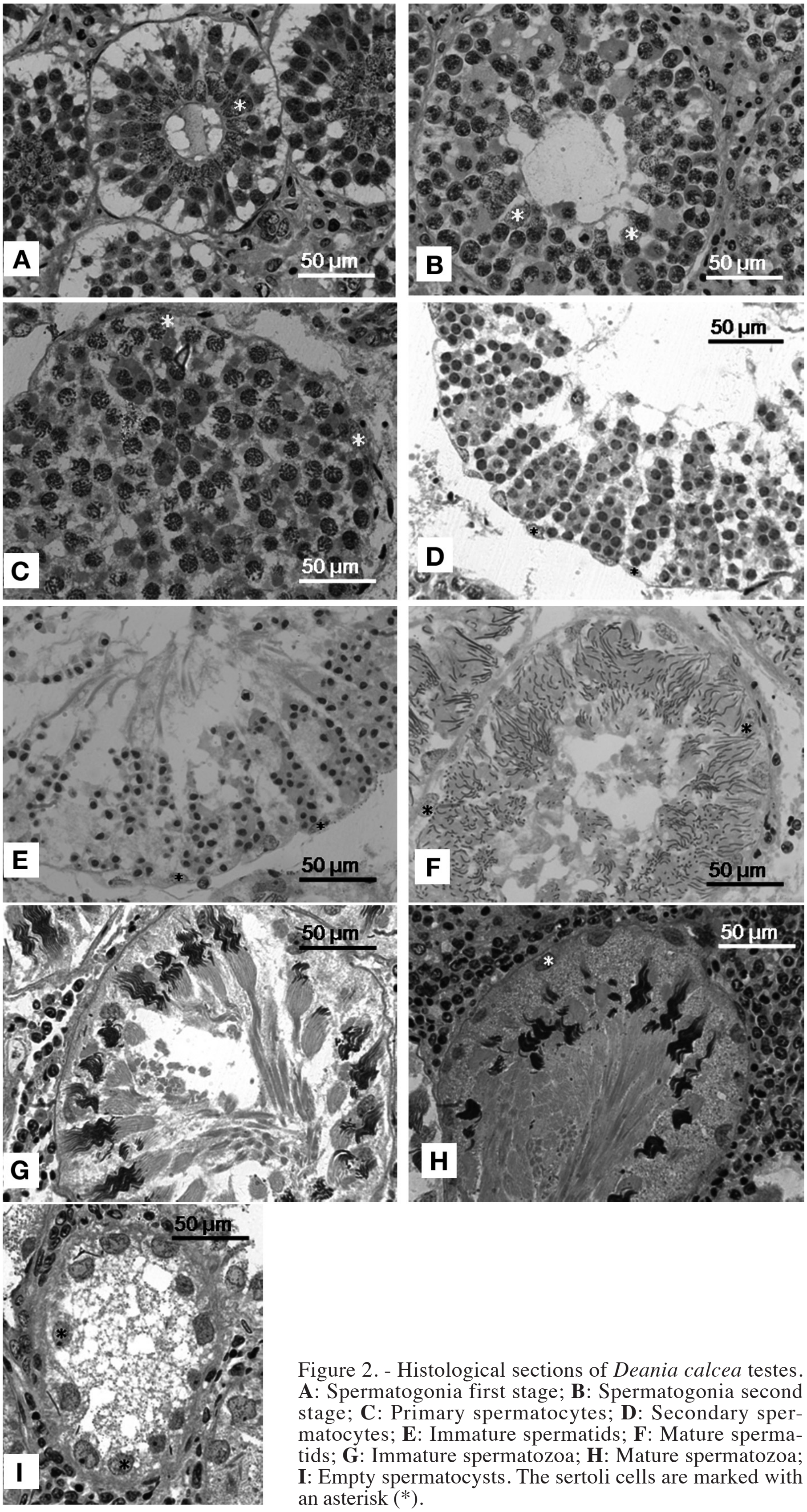

Figure 2. - Histological sections of *Deania calcea* testes. **A**: Spermatogonia first stage; **B**: Spermatogonia second stage; **C**: Primary spermatocytes; **D**: Secondary spermatocytes; **E**: Immature spermatids; **F**: Mature spermatids; **G**: Immature spermatozoa; **H**: Mature spermatozoa; **I**: Empty spermatocysts. The sertoli cells are marked with an asterisk (*).

Table II. - Number of *Deania calcea* specimens observed by sex and maturity stage from September 2008 to July 2009. * Maturity stages were not allocated to 20 individuals due to their poor freshness.

		Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	n
Females	**1**	32	17	21	13	20	16	22	15	19	23	10	208
	2	1		2	5	2	7		1	2	2	1	23
	3	1	1		17	3	4	2	2	1	5	4	40
	4	2			1		3	2	3	1	3	1	16
	5							1					1
	6	2	1		1	2	2	5	5		2	2	22
	7	4	2		1	2	2	2	3	2	1	2	21
	8	14	3	3	11	15	8	8	20	5	19	6	112
Males	**1**		2	2		1				6			11
	2	10	34	26		12	9	12		21			124
	3	1	4	3		3				5			16
	4									1			1
	n	67	64	57	49	60	51	54	49	63	55	26	595

Reproductive indices

The evolution of the female GSI by maturity stage is shown in figure 3A. The minimum value was obtained in stage 1 and the maximum in stage 3. This was an expected pattern since stage 1 corresponds to the females in the beginning of their maturity cycle and stage 3 is characterized by the ovaries with more advanced oocytes (Ø = 39.35 mm, on average), which are going to be fertilized in a near future. The Kruskal-Wallis test showed significant differences ($H_{(6, N = 341)} = 247.4$; $p < 0.001$) in the GSI values according to the different female maturity stages; the same trend was obtained after removing stage 3, which presented the highest GSI value ($H_{(5, N = 309)} = 196.0$; $p < 0.001$).

The OGWI evolution by maturity stage is shown in figure 3B. The highest values were obtained in maturity stages 3 and 4 and from this stage onwards, and during the uterine phase, a gradual reduction in the thickness of the oviducal gland was observed. The length of villi in the uterus by maturity stage is shown in figure 3C where we could observe that a gradual increase in the villi length occurred until stages 6 and 7, and decreasing after birth. Furthermore, a gradual enlargement of the uterus during the gestation period (stages 4, 5 and 6) was also observed and, after birth, the width of the uterus decreased (Fig. 3D).

In order to see if there was a seasonality of reproductive events, the monthly evolution of GSI of the ovarian cycle and OGWI were also analysed (Fig. 4). The GSI of immature individuals did not present significant differences along the year but GSI of mature specimens showed two different modes: one in March (smaller) and another in September (more evident). This pattern was also observed in the OGWI evolution with a small peak in February and a larger one in June.

The variation of the male GSI by maturity stage (Fig. 5) showed a minimum value at stage 1 and a maximum at stage 3. ANOVA analysis revealed statistically significant differences ($F = 23.6$, $p < 0.001$) in GSI values through the maturation stages; *a posteriori* Tukey test showed that at stage 2 GSI was not significantly different from the one at stage 3 GSI.

Fecundity

The OF ranged from 7 to 17 (12.30 ± 4.93) follicles per mature female. The UF was only estimated in stages 5 and 6, the two stages in which females presented embryos. The UF values varied between 11 embryos in stage 5 (corresponding to a single female sampled) and being lower at maturity stage 6 (4.01 ± 1.37 embryos per pregnant female).

A total of 72 embryos were sampled, with total length varying between 96 and 309 mm, with the embryos of smaller and larger dimensions being caught in the same month (March). Thirty-one embryos were females and 30 were males, indicating a sex ratio of 1:1 ($\chi^2 = 0.02$; $p < 0.8981$). It was not possible to assess the sex in none of the 11 embryos

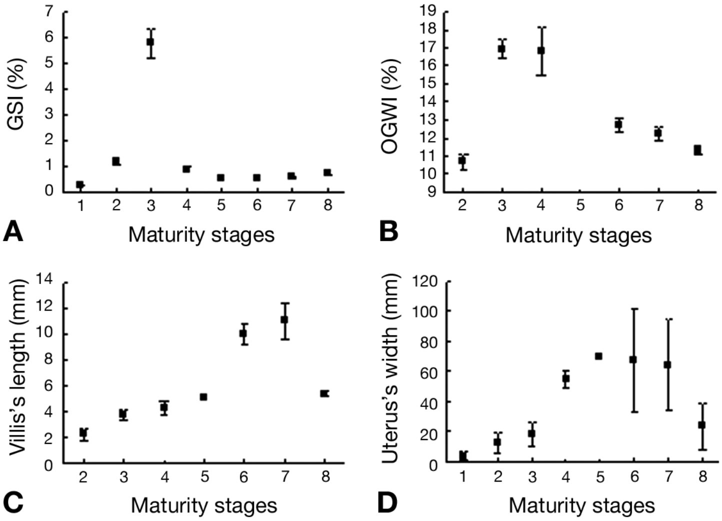

Figure 3. - Mean and standard deviation. **A**: GSI; **B**: OGWI; **C**: Length of villi; **D**: Uterus width by maturity stages of *D. calcea* females.

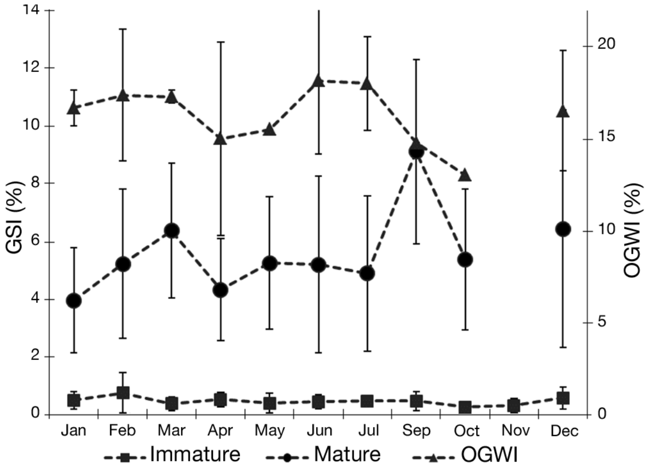

Figure 4. - Monthly evolution of GSI (immature and mature individuals) and OGWI during the ovarian cycle (mean ± standard deviation).

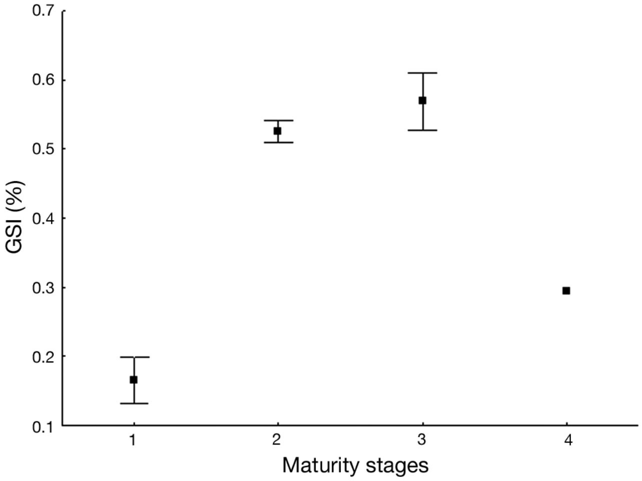

Figure 5. - Mean and standard deviation of GSI males by maturity stages in *Deania calcea*.

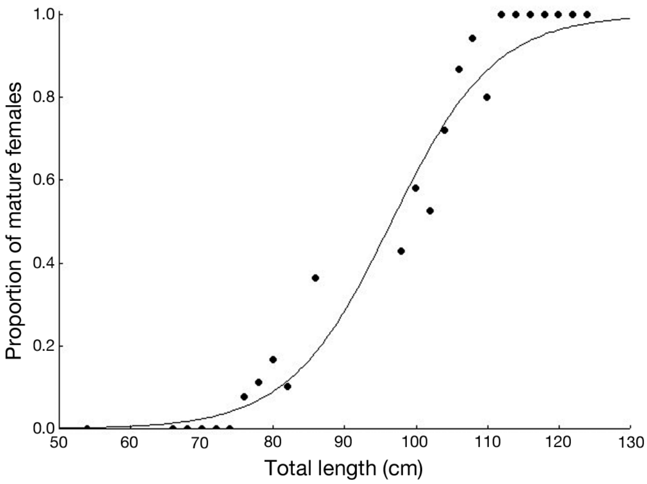

Figure 6. - Length maturity ogive in females of *Deania calcea*.

of the females in stage 5 because sex was not visible. The embryos of females in stage 5 and 6 showed clearly different characteristics: the smaller (embryos of females in stage 5) showed reduced pigmentation, no dorsal spines or teeth and had a large external yolk-sac with an average weight of 67.48 g; embryos of females in stage 6 were larger and well pigmented, presented dorsal spines, forming dental plaques, and had a smaller external yolk-sac with an average weight of 28.59 g.

Length at first maturity and length at birth

The length at first maturity estimated for females was 96.6 cm (r = 0.984) (Fig. 6). Insufficient information regarding males prevented the estimation of length at first maturity. The smallest distinguishable embryo measured 96 mm, and the fully developed embryo was 309 mm long. Embryos considered to be fully developed showed the external yolk sac totally depleted and ranged between 251 and 309 mm.

DISCUSSION

Sex ratio

Females were larger than males which can indicate a sexual dimorphism regarding TL. A lower abundance of males in all months sampled was also observed which may be related to a possible sex segregation in depth and/or geographical area as observed by Sousa *et al.* (2008) when studying the close species *Deania profundorum* (Smith & Radcliffe, 1913) in the Madeira archipelago and Clarke *et al.* (2001) in *Centrophorus squamosus* off Irish and Scottish waters. Because females are larger than males, and also due to size segregation among birdbeak dogfish, there is a preference of fishermen for females, leading to a higher percentage of females in landings. In fact, the sex ratio suggests that either female are more common in the population or they are more vulnerable to fishing than males. This bias in the landings should be considered in interpreting the results and must be taken into account in the future management of this species because the selective removal of females may have a disruptive effect on the reproductive output of the population (Braccini *et al.*, 2006).

Clarke *et al.* (2002) suggested that the existence of possible migration between the Irish waters and the Iberian waters was due to the fact that Irish samples were mainly constituted by adult fishes while the Portuguese samples (Machado and Figueiredo, 2000) were dominated by smaller fish. The current study shows that Portuguese waters are not dominated by small specimens as the work of Machado and Figueiredo (2000) might suggest. These authors captured small specimens (up to 86 cm) but their samples were obtained in bottom trawl research surveys while samples from the present study are from commercial landings (longline). This gear effect may explain the differences in lengths obtained for the two studies in the same area. Therefore, we believe that there is no evidence to confirm (or deny) the existence of possible migrations between the Iberian peninsula and the Irish waters and further studies should be implemented to clarify this issue.

Characterization of reproductive tract

Few studies have characterized the chondrichthyan reproductive tract, namely the changes in the ovaries, oviducal gland and uterus with maturity and even those that have tried to do it found difficulties in making direct comparisons (Storrie, 2004) because there is not uniformity of the maturity stage nomenclature.

The ovarian follicle appeared completely formed in a very early stage of the birdbeak dogfish ovarian development which led to the conclusion that the development of ovarian follicles is more related to the size of the follicles than to the maturity stages.

The morphology and functionality of the oviducal gland, present in all elasmobranch species, is specific to the species (Hamlett *et al*., 1998) and one or two zones among the four identified zones in the typical oviducal gland may be absent. In fact, the histological observation of the oviducal gland of the birdbeak dogfish didn't present the papillary zone in opposition to *Urolophus jamaicensis* (Cuvier, 1816) in which the baffle zone is lacking (Hamlett *et al*., 1998).

The shape and size of uterine villi reached their maximum development at the gestation stage, as already observed by Girard and Du Buit (1999) in two aplacental viviparous deep sea sharks, *Centrophorus squamosus* and *Centroscymnus coelolepis*. The increased vascularization (both central and peripheral) present in the later stages of pregnancy can be due to the increase in respiratory and excretion exchanges between the mother and the embryos, as stated by Storrie (2004) who also found numerous blood vessels that increased in size in the uterine mucosa of late term *Mustelus antarcticus* Günther, 1870. The presence of secreting cells in the uterine villi may be related to a matrotrophic strategy (Paiva *et al*., unpubl. data).

Among the three structural types of elasmobranch testes described by Pratt (1988), birdbeak dogfish testes belong to the diametric type as other Squaliformes and Carcharhiniformes. The spermatogenesis is similar to what happens in other vertebrates. Spermatocysts, the basic unit of evolution in the chordate testis (Parsons and Grier, 1992), are in birdbeak dogfish a closed spherical entity bounded by a basement membrane that gets together several spermatoblasts, i.e. a set of a single Sertoli cell and its associated germ cells (Hamlett, 1999). Several stages have been ascribed to the development of the spermatocysts, from seven stages described by Parsons and Grier (1992) to 18 described by Mellinger (1965). In this paper we considered eight stages. The main difference to the Parsons and Grier (1992) proposal is an additional stage in the evolution of spermatids, since we have considered the presence of immature and mature spermatids because a clearly morphological distinction among them could be observed. Immature spermatids presented the ellipsoid nucleus but no bundle appearance, typical of mature spermatids.

Reproductive cycles

Regarding the female reproductive cycle, this study adopted an eighth stage maturation scale (Figueiredo *et al*., 2008), instead of seven stages as suggested by Stehmann (2002), since differences in the reproductive structures of females maturing for the first time (classified as being in stage 2) and those that have matured more than once (classified in the new scale as being in stage 8) were observed. These two stages, together with stage 1, were present in all samples, meaning that for a large time period, there is some inactivity in both ovarian and uterine cycles.

The low percentage of uterine stages found in the present work can be related with a possible preference for other depths by pregnant females or with the existence of a migratory behaviour to breeding areas. A small number of gravid females was also reported by Clarke *et al*. (2002) who noted a tendency for these specimens to occupy shallower depths than immature females. It is important to stress that these authors did not find newborn specimens in the Irish waters, and this was associated to the low frequency of uterine stages in both Iberian and Irish waters supporting the idea of a possible breeding area still unknown where would congregate specimens from different European regions. Furthermore, this segregation by reproductive stage was documented for other deep-sea sharks and may be considered as an adaptation to the constrained environment in which these species live (Figueiredo *et al*., 2008).

Very recently there was an attempt to establish a uniform terminology for the different phases of the reproductive cycle for both male and female elasmobranch and teleost fishes as was suggested by Brown-Peterson *et al*. (2011). The eight stages maturity scale used in our study can be easily converted into the six maturity stages suggested in the new terminology (Tab. I). As it can be observed, the most important and significant difference in the females' maturity scale relied on grouping the three first uterine stages (4, 5 and 6) into a unique one (actively spawning capable). Regarding the males, we found no evidences that indicated the presence of resting or regenerating stages.

Reproductive indices

Female GSI presented the expected trend with the higher values corresponding to the ovaries with the larger oocytes (stage 3) ready to be fertilized. Therefore, this same stage presented the higher OGWI value as the larger oocytes must pass through the oviducal gland where they will be fertilized. From this stage onwards, and during the uterine phase, a gradual reduction in the thickness of the oviducal gland is observed until achieving very low and similar values to those presented in stage 2. There is a graphical evidence for the existence of two periods of higher ovulation (March and September). The greatest values of oviducal gland width preceding the ovulation also support this hypothesis since the oviducal gland must reach its largest dimension to allow the passage of the follicle towards fertilization.

Like other sharks, the paired uteri of *Deania calcea* are both functional and they share similar characteristics with other Chondrichthyes. The uterus expands with maturity, the diameter increases as pregnancy proceeds and regenerates after birth. This is the preparation for the next pregnancy, as observed by Storrie *et al*. (2009) in *Mustelus antarcticus*. However, we verified that villi gradually increased in size until stages 6 and 7, decreasing after birth. This is naturally related to the increase of the intra-uterine metabolic activity between mother and embryos.

Fecundity

Regarding fecundity of *D. calcea*, the OF values obtained in this work – 7 to 17 follicles per mature female – are similar to those reported by Yano (1991) – 19 follicles per mature female – and Clarke *et al*. (2002) – 6 to 14 follicles per mature female. As to the UF estimates, the values obtained in the present work showed a marked reduction on the number of embryos from the maturity stage 5 to 6, which is related to the handling procedures of pregnant females during the catch and storage of the individuals at the ship deck by the fishermen, leading to the loss of some embryos. Nevertheless, the UF estimates for stage 5 female – 11 embryos – is similar to those reported by Yano (1991) – 12 to 23 embryos – and Clarke *et al*. (2002) – 8 to 14 embryos.

Length at first maturity and length at birth

The length at first maturity estimated for females was 96.6 cm, which is similar to that determined by Yano (1991) in the southeast Atlantic of Namibia, but lower than the one (105 cm) obtained for Japanese (Yano, 1991) and Irish waters (Clarke *et al*., 2002).

The largest fully developed embryo with the external yolk sac totally depleted was 309 mm. Although no free swimming newborn was sampled, this value was considered to be an estimate of birth's length and is close to the one reported by Yano (1991) as the smallest free swimming specimen with 312 mm.

In conclusion, this paper provides valuable information on the birdbeak dogfish reproductive parameters, a commercially exploited species in Portuguese waters. The possible size segregation or a preference of fishermen for females (leading to a higher percentage in landings) makes them more vulnerable to fishing than males. The characterization of the reproductive tract associated with the definition of the reproductive cycle, both by direct and indirect observations (indices) and the estimation of the reproductive parameters (fecundity and length at the first maturity), provides useful information to be used in future assessment models in order to implement a management plan towards the sustainability of fishing resource.

Acknowledgements. - We would like to thank 'ArtesanalPesca' (Sesimbra, Portugal) for their support in providing the birdbeak dogfish specimens used in this work.

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Reçu le 3 mai 2010.
Accepté pour publication le 19 août 2011